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Simulated moult reduces flight performance but overlap with breeding does not affect breeding success in a long-distance migrant

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Abstract

1. Long-distance migrants are time-constrained as they need to incorporate many annual cycle stages within a year. Migratory passerines moult in the short interval between breeding and migration. To widen this interval, moult may start while still breeding, but this results in flying with moulting wings when food provisioning.
2. We experimentally simulated wing gaps in breeding male pied flycatchers by plucking two primary feathers from both wings. We quantified the nest visitations of both parents, proportion of high-quality food brought to the nestlings and adults and nestlings condition. Differences in oxidative damage caused by a possible reduction in flight efficiency were measured in amounts of ROMs and OXY in the blood. We also measured how flight performance was affected with recordings of the male`s escape flight using high-speed cameras. Finally, we collected data on adult survival, clutch size and laying date in the following year.
3. "Plucked" males travelled a 5% shorter distance per wingbeat, showing that our treatment reduced flight performance. In line with this, "plucked" males visited their nests less often. Females of "plucked" males, however, visited the nest more often than controls, and fully compensated their partner's reduced visitation rate. As a result, there were no differences between treatments in food quality brought to the nest, adult or chick mass or number of successfully fledged chicks. Males did not differ in their oxidative damage or local survival to the following year. In contrast, females paired with plucked males tended to return less often to breed in the next year in comparison to controls, but this difference was not significant. For the birds that did return, there were no effects on breeding.
4. Our results reveal that wing gaps in male pied flycatchers reduce their flight performance, but when it occurs during breeding they prioritise their future reproduction by reducing parental care. As a result, there is no apparent detriment to their condition during breeding. Because non-moulting females are able to compensate their partner's reduced care, there is also no immediate cost to the offspring, but females may pay the cost suffering from a reduced survival.

KEYWORDS
Ficedula hypoleuca, high-speed camera, oxidative stress, parental care, pied flycatcher, PIT-TAG, plumage, trade-off

1 | INTRODUCTION

One of the major assumptions in life-history theory states that there is a trade-off between current and future reproductive success (Stearns, 1992; Williams, 1966). This means that an increased breeding effort leads to a decreased survival or less successful breeding in the following season possibly caused by a depletion of available energy resources (Stearns, 1992; van Noordwijk & de Jong, 1986). Birds have been typically used as model species in life-history studies because many types of manipulations on breeding effort can be performed (Sanz, 1997; Siikamäki, Hovi, & Rätti, 1994; Verhulst & Nilsson, 2008; Visser & Lessells, 2001). However, even in birds, a physiological mechanism mediating the trade-off between current and future reproductive success remained elusive for a long time (Williams, 2012). Because immediately after breeding, most Passeriformes from temperate regions undergo an extensive replacement of their feathers, i.e. moult (Jenni & Winkler, 1994), and because renewed feathers are important for future survival (Nilsson & Svensson, 1996), it was suggested that moult could be a mediator of this classical trade-off (Hemborg, Sanz, & Lundberg, 2001; Nilsson & Svensson, 1996; Siikamäki et al., 1994; Williams, 2012). Support for this hypothesis was offered by experiments in Eurasian blue tits (*Cyanistes caeruleus*), where delaying breeding decreased the quality of their body plumage quality and thus reduced their insulation capacity and, consequently, their winter survival (Nilsson & Svensson, 1996).

Moult is an important annual cycle stage in which birds replace body and in some cases wing feathers (Jenni & Winkler, 1994). It is a supposedly energetically expensive stage, because small passerines may renew up to 25% of their lean dry mass in feathers (Jenni & Winkler, 1994; Murphy & King, 1991, 1992). Moreover, flight and thermoregulation might be compromised during moult stage due to gaps in the plumage (Hemborg & Lundberg, 1998; Jenni & Winkler, 1994; Swaddle, Witter, Cuthill, Budden, & McCowen, 1996). To cope with such challenges, species resort to distinct moult strategies, such as the temporal separation of moult from other annual cycle stages, like migration (Barta et al., 2006, 2008; Jenni & Winkler, 1994; Wingfield, 2008). Although moult needs to be completed within a certain time (e.g. before conditions in the environment deteriorate), birds are also constrained in how fast they can moult. For example, speeding up moult may negatively affect plumage quality for both flight and body feathers (Dawson, Hinsley, Ferns, Bonser, & Eccleston, 2000; Vágási et al., 2012). Therefore, the timing of moult in the annual cycle of birds is a decision with large fitness consequences.

Long-distance migrants are particularly time-constrained due to their need to incorporate many life cycle stages within a single year (Both & Visser, 2001; Hall & Fransson, 2000; Jacobs & Wingfield, 2000; Wingfield, 2008), meaning less time available for moult (Barta et al., 2008; Hemborg et al., 2001; Holmgren & Hedenström, 1995; Jenni & Winkler, 1994). Replacing flight feathers during migration is regarded as a highly expensive strategy (Jenni & Winkler, 1994; Merilä, 1997; Pulido & Coppack, 2004). Thus, for these birds, moulting while still rearing chicks (moult-breeding overlap) could serve as a time-saving strategy if costs of moult migration or delayed migration surpass the potential moult-breeding overlap costs (Hemborg et al., 2001; Merilä, 1997). Despite moult-breeding overlap being considered a costly strategy, the occurrence of moult-breeding overlap is relatively common (Echeverry-Galvis & Hau, 2013; Hemborg, 1998; Hemborg & Merila, 1998; Jenni & Winkler, 1994). Therefore, for long-distance migrants, the trade-off between current and future reproductive success could also be mediated by moult (Siikamäki et al., 1994). With the distinction that for migrants the trade-off involves moult timing and flight feather condition for migration (instead of body feather condition for winter insulation in non-migrants; Nilsson & Svensson, 1996).

We are left with the question of how costly it is to moult while breeding. The moult-breeding trade-off has previously been examined in different studies with a particular focus on the migratory pied and collared flycatchers (Table 1). In several cases, the aim was to understand how a costly breeding would affect moult onset (e.g. cause a delay) and its related fitness consequences. This was done by (1) manipulating clutch (Sanz, 1997; Siikamäki et al., 1994) or brood sizes (Hemborg & Merilä, 1999), which increases parental care, at least for females; (2) manipulating timing of breeding, forcing delayed birds to overlap moult and breeding or to delay their moult (Hemborg, 1998; Siikamäki, 1998); (3) lifting breeding constraints with food supplementation (Moreno, Merino, Potti, de León, & Rodríguez, 1999; Siikamäki, 1998); (4) by looking at the natural variation (Hemborg & Merila, 1998; Hemborg et al., 1998; Hemborg et al., 2001; Hemborg, 1999; Morales et al., 2007); (5) using a more direct method to simulate moult-breeding overlap, by plucking feather to create a wing gap (Hemborg & Lundberg, 1998). Due to the different purposes of the studies 1–4, it is not possible to distinguish between the effects of moult-breeding overlap per se and other factors, such as time in the season, individual quality or change in energy balance.

The moult-breeding overlap can also be examined from different angles. For example, the gaps in the wing might reduce flight performance at a time when birds need to actively provide food for the young. This increases energetic costs during foraging flight (Sanz, Kranenbarg, & Tinbergen, 2000; Winkler & Allen, 1995), might reduce prey capture performance on the wing (Navarro & González-Solís, 2006) and negatively affect escape performance from predators (Echeverry-Galvis & Hau, 2013). We could also expect a sexual conflict, because it is usual for males, but not females, to overlap moult and breeding, suggesting that males will be more likely than females to prioritise future reproduction (Harrison, Barta, Cuthill, & Székely, 2009; Hemborg,

1999; Hemborg & Merila, 1998; Williams, 2012). Finally, because moult and breeding are supposedly equally demanding stages, if the individuals still allocate many resources to breeding even if moulting, fewer resources will be available to the newly produced feathers. This would result in lower quality feathers for the consecutive migration (Echeverry-Galvis & Hau, 2013).

A common point of the examples above is the assumption that replacing feathers during moult is not only energetically expensive by itself but that it also negatively affects the flight capabilities of the animal (Hemborg & Lundberg, 1998). This was supported by a number of studies in laboratory conditions that quantified the effects of moult on flight performance (Chai, 1997; Swaddle & Witter, 1997; Swaddle et al., 1996; Williams & Swaddle, 2003), suggesting that wing gaps could be detrimental to the individual, for example, in terms of predation pressure (Lind, Jakobsson, & Kullberg, 2010; Swaddle & Witter, 1997). Curiously, this ends up generating a segregation: because fitness should be investigated in field conditions while flight dynamics studies require a controlled environment, fitness costs of moult-breeding overlap have been looked at in the field assuming a locomotion cost (Table 1; see also Echeverry-Galvis & Hau, 2013 for an exception), and effects of moult on locomotion have been quantified in laboratory settings (see McFarlane, Altringham, & Askew, 2016 for a field example) assuming a fitness cost to the individual bird, outside the context of moult-breeding overlap.

Therefore, in the present study, we attempted to integrate these two approaches by (1) using an experimental manipulation that only tests for the effects of simulated moult-breeding overlap, creating wing gaps while controlling for other factors; (2) collecting field data on parental care, breeding success and carry-over effects to assess fitness consequences; (3) quantifying the effects of the wing gaps on escape flight to assess the magnitude of their effect on flight performance; (4) measuring oxidative stress as an integrative physiological trait representing life-history trait costs or constraints (Monaghan, Metcalfe, & Torres, 2009); (5) performing all the previous procedures on the same individuals, ensuring the integration of the different components. Thus, by broadly looking at parental care, condition, locomotion and reproductive success in an experimental context, we aimed at disentangling which aspects were affected by wing gaps during breeding.

2 | MATERIALS AND METHODS

2.1 | Experimental design and field data collection

The experiment was conducted with the nest box breeding population of pied flycatchers *Ficedula hypoleuca* ([Pallas], 1764), in the Hoge Veluwe National Park (see Appendix S1, Supporting Information for more information). All procedures were carried out under licenses of the Animal Experimental Committee of the Royal Netherlands Academy of Sciences (KNAW) (protocol NIOO 14.13).

Nest boxes were checked once to twice per week as soon as pied flycatcher nesting material was detected. After the first eggs were found, nests were checked more frequently to determine the total clutch size, incubation onset and hatching date. When chicks were 6 days old, we paired nests based on their brood size and consequently on chick hatching date, and randomly selected pairs of nests to be part of the experiment. For each pair, one nest was randomly assigned to the "control" group and the other to the "plucked" experimental group. Pairs were created throughout the season trying to span a large range of hatching dates.

When chicks were 7 days old, we captured the adult birds with spring traps installed at the nest box entrances. They were identified with aluminium rings (if not already ringed) and a ring with a uniquely coded PIT-TAG transponder. We then took measurements of their tarsus and third primary length (to the nearest 0.1 mm) and weight (to the nearest 0.01 g). Finally, if a male was in the "plucked" group, we simulated early moulting stages by plucking two of their innermost primary feathers. For all cases, we removed primary 2 and 3, following the moult sequence. We did not remove the first primary because this allowed us to assess the date of the onset of natural moult as the moment when the first primary was dropped. This procedure was similar to (Hemborg & Lundberg, 1998), however, they plucked both males and females when chicks were 5 days old, whereas we let females untreated. Females and males from control groups were captured and handled in the same way, but we did not pluck feathers from them. After this procedure, all birds were immediately released close to their breeding nest boxes.

When chicks were 12 days old, adults were caught again, and we took a second weight measurement and removed the rings with the PIT-TAGS to prevent any effect of additional tags on the bird's migration. After this procedure, the females were immediately released, but the males were taken to the Netherlands Institute of Ecology and their flight performance was tested (see the laboratory experiments section below).

We measured the chicks' body mass when they were 7 days old (prior to the manipulation) and at 12 days old (after the manipulation) when we also collected data on tarsus and primary length. At the end of the season, nests were inspected for fledgling success by looking and identifying any dead chick(s).

2.2 | Nest visitations and prey choice

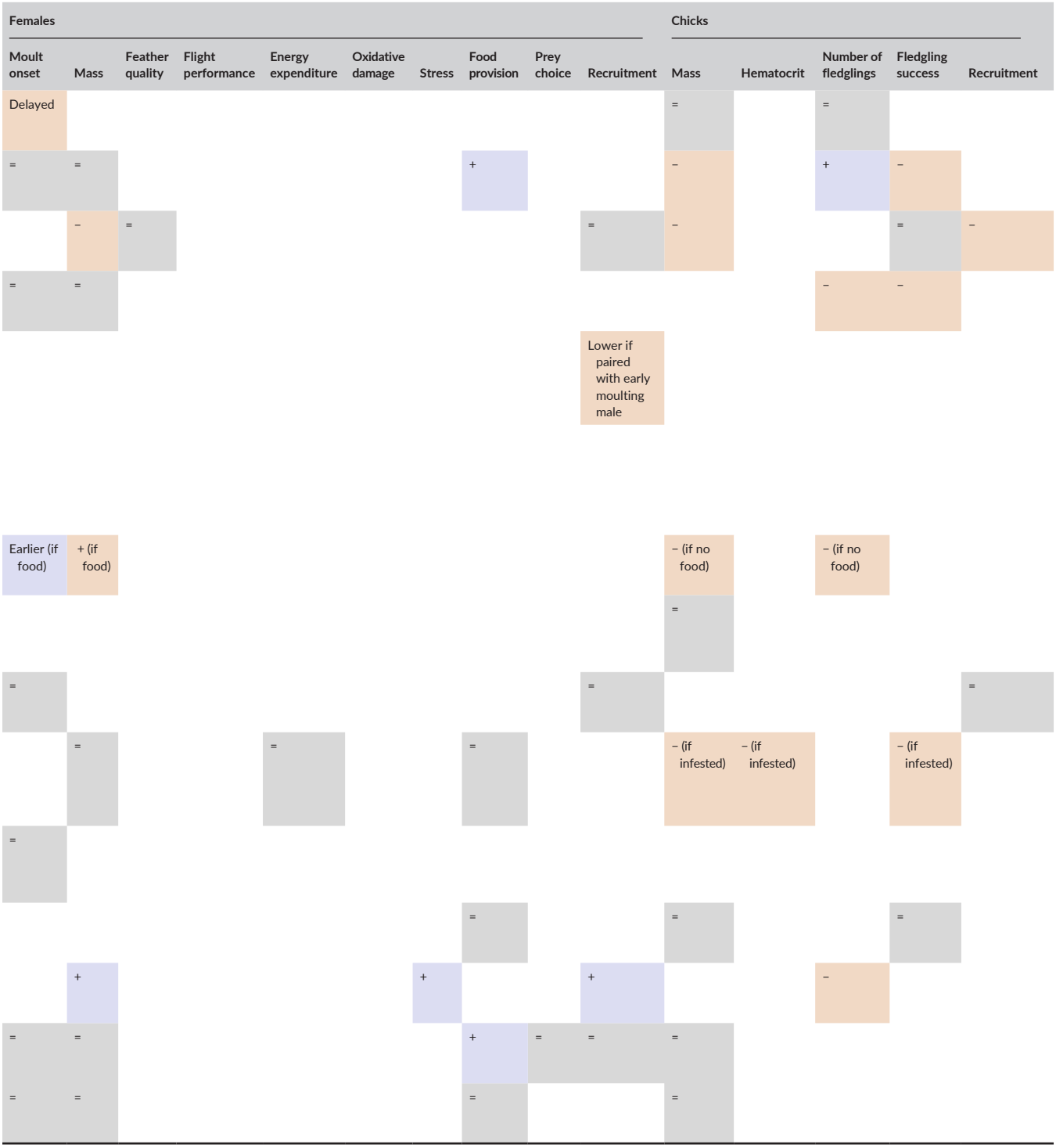
Data on prey choice was collected using cameras (mini CMOS camera, Velleman) installed in the nest box lids and then connected to video recorders (Archos 406 media player). Cameras were installed when chicks were 9 days old and recordings took place when chicks were 10 days old. We recorded 2 hr of visits per nest box starting at around 10.00 hr in the morning. Prey was then classified (to Order, also discriminating life-stage for Lepidoptera and Coleoptera) and we carried two separate analyses to look at the proportion of two high-quality food for pied flycatcher chicks: larvae of Lepidoptera and Coleoptera (henceforth "caterpillars") or spiders (Samplonius, Kappers, Brands, & Both, 2016).

When chicks were 9 days old, we also installed PIT-TAG readers at the entrance of each box to detect when each individual bird (equipped with PIT-TAGS) visited the nest. These measurements were

TABLE 1 Studies that investigated the trade-off between moult and breeding in pied or collared flycatchers (studies with collared flycatchers indicated by an * after year). Colours indicate direction of effects: red: delayed/later or lower; blue: advanced/earlier or higher; grey: no direction

Author(s) (Year)	Manipulation/Approach	Reference for the effect direction	Males								
			Moult onset	Mass	Feather quality	Flight performance	Energy expenditure	Oxidative damage	Food provision	Prey choice	Recruitment
Siikamäki et al. (1994)	Clutch size manip. (−2, −1, 0, +1, +2 eggs)/Test of reproductive effort on timing of moult.	Enlarged	Delayed								
Sanz (1997)	Clutch size manip. (−2, 0, +2 eggs)/Test of reproductive effort on timing of moult.	Enlarged	=	=					+		
Hemborg and Lundberg (1998)	Moult simulation (males and females)—feather plucking/Test of wing gap on fitness	Plucked		−	=						−
Hemborg (1998)	Timing manipulation/Test of termination of breeding on moult onset	Delayed	Delayed								
Hemborg and Merila (1998)*	Natural variation/Comparison between males and females		Earlier than females								No effect if early moult
Hemborg, Lundberg, and Siikamäki (1998)	Natural variation/Comparison between populations		Overlap larger than females, but similar across populations								
Siikamäki (1998)	Hatching date + food supplementation/Test of quality vs. timing	Delayed	Earlier (if food)	=							
Hemborg (1999)	Natural variation/Year to year comparison		Proportion of overlap varies, but duration not	=							=
Hemborg and Merilä (1999)*	Brood size manip. (−2, 0, +2)/Test of reproductive effort on timing of moult	Enlarged	=								=
Moreno et al. (1999)	Feather plucking (higher female effort), food supplementation (lower effort)/Test of female effort on fitness without changing brood size	Handicapped		=					=		
Hemborg et al. (2001)	Natural variation/Latitudinal comparison		Higher proportion in higher latitudes								
Sanz, Moreno, Merino, and Tomás (2004)	Immunization/Test if investment in immunity affect moult or breeding success in males	Immunized	Delayed	=					=		
Morales et al. (2007)	Natural variation/Effect of female moult breeding overlap and stress, parasites and condition	Overlap									
Tomotani et al. (2017)	Moult simulation (only males)—feather plucking/Test of wing gap on fitness	Plucked	=	=		−		=	−	=	=
Tomotani, de la Hera, et al. (Submitted)	Timing manipulation/Test of termination of breeding on moult onset		=	=	=				=		

taken continuously until chicks were 12 days old and parents were caught for the second time. Thus, in the same days that equipment was placed and removed (chicks 9 and 12 days old), the recordings did not cover the entire day and were thus discarded. The readers detect the presence of the transponder in the vicinity of the nest box every second. This means that if a bird is perched in the front of the nest box, the equipment will detect multiple records of it, even though the bird is not visiting the nest box multiple times. In order to remove those



pseudo visits, we used the video data collected for prey choice to discriminate the minimum time interval that actually reflected a real nest visitation. We then used this interval to filter all "visitations" with a shorter length of time.

2.3 | Flight trials and housing conditions

During the flight performance trials, males were housed in individual cages (90 cm × 50 cm × 40 cm), provided with live food (mealworms

and crickets) and water ad libitum. Every day, each bird was weighed and checked for moult. Conditions of light and temperature were kept as close to the natural situation as possible, with all windows open, allowing birds to be exposed to natural illumination conditions. This was supplemented by two fluorescent lamps connected to a timer that switched on half an hour after the sunrise and off half an hour before the sunset. Therefore, birds were exposed to the natural photoperiodic variation with natural duration of twilights. For the purpose of collecting precise moult data, we kept the birds for 14 days in captivity before releasing them back to the Hoge Veluwe study area, close to their breeding nest box. Most data collection relevant for this study, however, was done in the first 3 days after the bird arrived at the institute.

Two days after being taken indoors, males were tested in a vertical flight chamber in which they were filmed flying straight up using a stereoscopic high-speed videography system (Figure 1a). The system consisted of three synchronised high-speed cameras (Basler piA64-210gm) that filmed the flying birds in front of a black background at a spatial resolution of 648 × 488 pixels and a temporal resolution of 150 or 200 frames per second (Figure 1b, see Video S1 for more details). The cameras filmed the flying birds within a field of view at the bottom half of the flight chamber (Figure 1a), because we assumed that during the initial phase of the flight trajectory the bird would show maximum effort to accelerate up to the top perch, while during the second phase of the manoeuvre they would start slowing down to prepare for landing.

Every bird was always tested 2 days after arriving at the laboratory, thus exact days depended on when the individual birds bred. Per test, we recorded five to six consecutive flight manoeuvres, aiming to achieve at least one "straight" flight trajectory, whereby the bird would fly up using a mostly straight flight path. All analyses were based on the straight flight manoeuvres only. The whole data collection spanned 21 consecutive days in total, which was the same range of hatching dates of our experimental birds. Once a week, we calibrated the camera system using a DLT routine based on 22 objects randomly placed throughout the region-of-interest (Hartley & Zisserman, 2000).

2.4 | Flight performance components (see Appendix S1 for more details)

Throughout each flight trial, we determined the three-dimensional displacement of the tip of the beak and the start of each wingbeat using the tracking software developed by the Hedrick Lab (Hedrick, 2008). Based on these data, we determined for each flight trial the average wingbeat frequency f_{mean}, the average flight velocity U_{mean} and the average vertical velocity w_{mean}. By dividing U_{mean} and w_{mean} through the average wingbeat frequency f_{mean}, we determined the average distance travelled per wingbeat D_{mean} and the average height gained per wingbeat $D_{z,mean}$ respectively.

For flapping flight at low flight speeds, such as the vertical flight manoeuvres in our experiments, the distal part of the beating wing

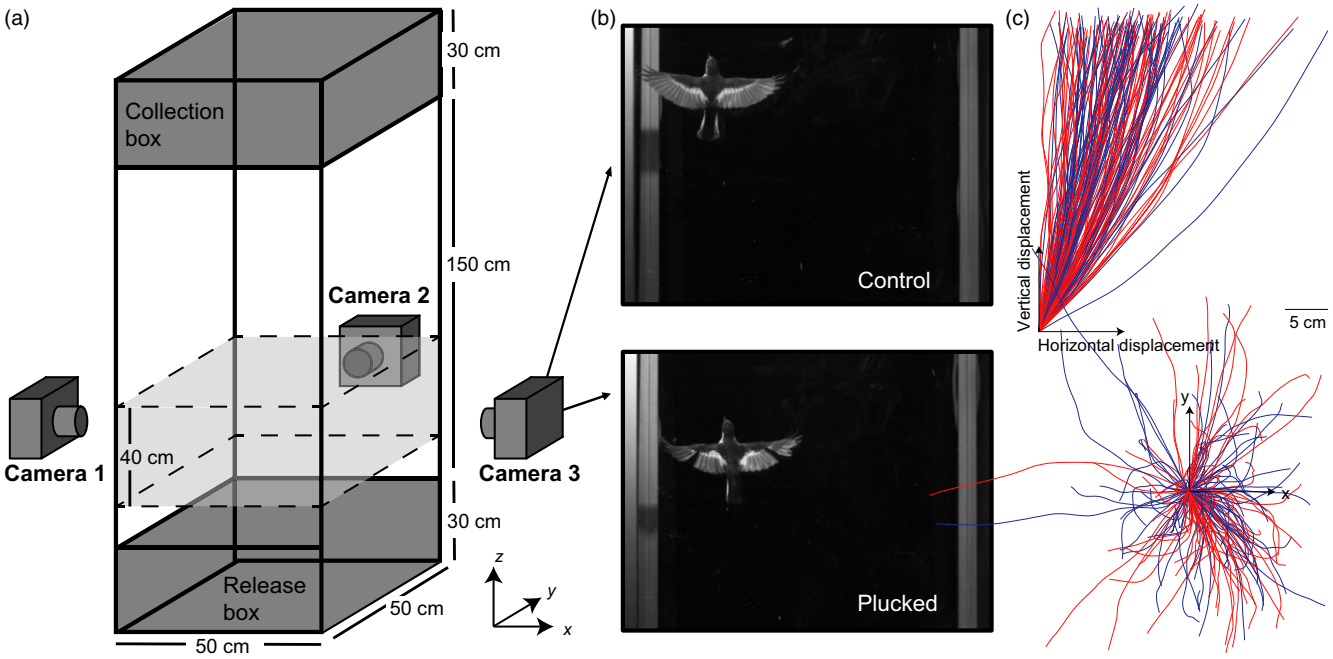


FIGURE 1 (a) Schematic view of the experimental setup for studying the effect of moult on flight performance, consisting of a vertical flight tunnel with a release box and a collection box, and three high-speed cameras for filming upward flight within an *c.* 40 cm high field of view (dashed box). (b) Two sample images from high-speed camera 3 of a control bird (top) and a plucked bird (bottom) in upward flight. For each individual bird, we used such images to estimate the second moment of wing area. (c) Three-dimensional trajectories of all vertical flights used in the analysis, whereby blue trajectories are of control birds and red trajectories are of plucked birds. The top panel shows vertical displacement vs. horizontal displacement (vector sum of displacement along the *x*- and *y*-axes), and the bottom panel shows the displacement along the *x*- and *y*-axis (see panel (a) for the coordinate system). All flight sequences were aligned such that the start of each trajectory is at the origin

moves faster than the proximal wing section, and therefore contribute more to aerodynamic lift production. For this reason, the aerodynamic forces produced by a beating wing at low flight speeds scale approximately linearly with the second moment of area S_2 of that wing (Muijres, Iwasaki, Elzinga, Melis, & Dickinson, 2016; Weis-Fogh, 1973). And thus, to quantify the effect of wing morphology on flight performance, we determined the second moment of area for both wings of each bird, based on the videography images. For each bird, we selected a video sequence image at which the wing was outstretched during the down stroke and positioned as perpendicularly as possible to the direction of view of the video camera (Figure 1). For that image, we traced the outline of the wing using Photoshop (Adobe, Inc), and based on the outline we determined S_2, using a custom-written MATLAB (Mathworks, Inc) program.

All relevant flight dynamics parameters were normalised using the mean value for all birds in the control group ($U^*_{mean} = U_{mean}/U_{mean,control}$, $w^*_{mean} = w_{mean}/w_{mean,control}$, $D^*_{mean} = D_{mean}/D_{mean,control}$, $D^*_{z,mean} = D_{z,mean}/D_{z,mean,control}$, $f^*_{mean} = f_{mean}/f_{mean,control}$ and $S^*_2 = S_2/S_{2,control}$).

2.5 | Blood sampling and biochemical analyses

Oxidative damage was used as a proxy for potential differences in effort between plucked and control birds after the flight trial. Samplings were only done after the end of the experiment to avoid additional burdens to the animals caused by sampling blood during the experiment. Thus, 1 day after the flight trial, males were blood sampled from the brachial vein. We collected circa 40 μl of blood; samples were immediately centrifuged and the plasma stored at −80°C.

The plasma oxidative damage was quantified by measuring the levels of reactive oxygen metabolites (ROMs) with the d-ROM test (Diacron International, Grosseto, Italy; Costantini et al., 2006). Reactive oxygen metabolites are hydroperoxides, end-products of the oxidation of lipids, proteins and nucleic acids. Since hydroperoxides can further generate reactive species via Fenton reaction, they also indicate potential future oxidative insult. The assay was performed on 4 μl of plasma and 100 μl of the reactive solution provided with the kit. The absorbance was read with a microplate reader (Multiskan Go, Thermo Fisher, Vantaa, Finland) at a wavelength of 505 nm in endpoint mode. Measurements were expressed as mmol*L^{-1} of H_2O_2 equivalents. All samples, 5-points calibration curve and controls for high and low concentrations were run in duplicate. The mean intraplate coefficient of variation (CV) of samples was 6.15%, while the interplate CV calculated from the standards was 4.32%.

The non-enzymatic antioxidants present in the plasma were quantified using the OXY-Adsorbent test (Diacron International, Grosseto, Italy) (Costantini et al., 2006). The assay quantifies the antioxidant capacity (OXY) of the sample exposed to the oxidant action of hypochlorous acid (HOCl). An aliquot of 2 μl of diluted plasma (1:100 with distilled water) was incubated at 37°C with 200 μl of HOCl solution for 10 min. The same volumes were used for the reference standards and blank (i.e. water). At the end of the incubation, 2 μl of the chromogen provided with the kit was added to each well and the absorbance read at 546 nm with a microplate reader (Multiskan Go, Thermo Fisher Scientific, Vantaa, Finland). The OXY was expressed in μmol HClO × ml^{-1}. All samples, standards and blank were run in duplicate (intraplate CV of samples was 7.31%; interplate CV of standards was 3.15%).

2.6 | Carry-over effects

One year after the experiments we recovered 14 males and 19 females, breeding in our study area, that were part of the experiment. We used this information to calculate the proportion of (locally) surviving birds from each treatment and, in the case of the females, we also obtained the next year laying dates and clutch sizes. The male comparison, however, need to be treated with care, because mostly males from the control group were deployed with geolocators after the experiment ended (24 controls and 5 plucked, for purposes not related to the present experiment, Tomotani, van der Jeugd, et al. (2017)), which could impose an additional constraint to the survival probability for this group (Bowlin et al., 2010). The male survival comparison is still relevant since previous geolocator studies with pied flycatcher reported low impact on return rates of tagged birds in comparison to controls (Ouwehand & Both, 2017).

2.7 | Data analysis

We used either mixed effect models or multiple regressions to test for treatment effects, all analyses were performed in R version 3.2.1 (R Core Team, 2015), using "LME4" and "PBKRTEST" R packages (Bates, Maechler, Bolker, & Walker 2015; Halekoh & Højsgaard 2014). To define the minimal model, we used backward model selection, dropping non-significant terms in each step.

Adult and chick mass were analysed in relation to treatment, tarsus length and brood size in the case of chicks or sex in the case of adults. We included the breeding nest box (henceforth "box," which is composed of the two breeding birds and their chicks and has only one treatment) nested within experimental pair (henceforth "pair," which contains two boxes, one from each treatment that have a similar hatching date and brood size as described in the experimental design) as a random effect. Number of fledged chicks was also analysed in relation to treatment, hatching date and brood size, also including "pair" as a random effect.

Number of visits and proportion of caterpillars or spiders were tested in similar mixed effect models with the individual adult bird (henceforth: "individual" that allowed the inclusion of multiple observations from the same bird) nested within "box" and "box" nested within "pair" as random effects. For the number of visits, we tested the effect of treatment, sex (of the adult), chick age, hatching date and we also included all interactions between treatment, sex and chick age. For the proportions of high-quality prey, we included treatment, sex, hatching date and their interactions as fixed effects. To assess how much females compensate for reduced male visit rates, we also tested whether the sum of the male and female visits differed depending on treatment, also including "box" nested within "pair" as a random effect.

The normalised flight components total flight speed, vertical flight speed, distance travelled per wingbeat, height gain per wingbeat and

wingbeat frequency were analysed with "individual" as random effect and with treatment, second moment of wing area and their interaction as fixed effects.

The effect of treatment on the two oxidative damage measures: ROMs and OXY was tested using multiple regressions and also including the second moment of wing area, sampling date and their interactions with treatment.

The effect of treatment on the proportion of females that returned as breeders was tested in a logistic regression and multiple regression for next year laying date and clutch size. In all cases, we also included the previous year brood size at day 6 and previous year hatching date of the chicks. For the male recruitment, we also included whether the bird was equipped with a geolocator or not.

3 | RESULTS

Pairs of control and experimental nests (n = 29 pairs, i.e. 58 nests) with the same hatch date and same brood size were selected throughout the season to cover the full range of the hatching dates (range of experimental nests was 21 days, full range of all nests was 30 days, only very late broods were not selected). In some cases, we had to allow 1 day (four pairs) or one chick (10 pairs) of difference in a pair since there were not enough identical nests every day to select a pair of nests. We ensured, however, that the treatments were not significantly different in neither average chick hatching date ($F_{1,56}$ = 0; p = 1.0; control: 54.59 ± 4.99; plucked: 54.59 ± 4.91) nor in brood size ($F_{1,56}$ = 0.14; p = .71; control: 6.41 ± 0.68; plucked: 6.34 ± 0.72).

From the 58 nests used, two males were already moulting before the start of the experiment (one in the control and one in the plucked group), and these nests had to be excluded from further analysis. Later in the season, three birds from the plucked group (two males and one female) deserted their nests a few days after the treatment was applied. Finally, we lost some data due to equipment failure. Therefore, there is some variation in sample sizes in our field data: number of visits at day 10 was based on 51 nests; number of visits at day 11 was based on 53 nests; prey choice was based on 48 nests, adult condition was based on 53 males and 53 females; chick condition was based on 53 nests.

3.1 | Treatment effects

Our treatment in which the second and third primary feathers were removed in the plucked group reduced the second moment of wing area during flight by on average 28% relative to the control group ($F_{1,39}$ = 23.44; p < .01; $S_{2,\text{mean,control}}$ = 1.15 ± 0.21 × 10^{-6} m^4 (M ± SD) n = 22; $S_{2,\text{mean,control}}$ = 0.83 ± 0.18 × 10^{-6} m^4, n = 19). Natural moult onset was not affected by treatment ($F_{1,38}$ = 0.001; p = .98; control = 75.77 ± 1.02; plucked = 75.81 ± 1.08, in April days), moreover, plucked feathers only started re-growing once birds dropped their first innermost primary feather, which in most cases happened several days after the laboratory flight trials ended. Thus, our manipulations affected wing morphology without necessarily affecting energetic allocation on feather synthesis.

3.2 | Breeding data

There was no significant effect of treatment on adult or chick mass (we report the full statistics for each term at the point of exclusion from the model and the variance components of the random effects in the Appendix S1: Tables S1 to S6). The number of fledged chicks also did not differ between groups (Table S1) and is only dependent on the original brood size ($F_{1,36.89}$ = 9.80; p <.01; slope = 0.73 ± 0.23). Adult mass was significantly related to sex, females lost weight from day 7 to day 12 independent of treatment; weight of males either did not change or slightly increased ($F_{1,50.35}$ = 36.58; p < .01; females = −0.41 ± 0.07; males = 0.07 ± 0.07). There were also no significant differences between control and plucked males in their change in mass from the day when they were taken to the laboratory (chick-day 12), and the test day (chick-day 14) ($F_{1,23.26}$ = 0.41, p = .53, control males = −0.39 ± 0.65; plucked males = −0.26 ± 0.40).

The number of visits to the nest was related to sex and treatment, with a significant interaction between sex and treatment ($F_{1,51.02}$ = 5.79; p = .02): thus males and females respond differently to the treatment, with males in the plucked group decreasing the number of visits while females increase. The difference between plucked and control when each sex is tested separately (post hoc) is not significant (Table S1). The increased number of visits of plucked females (9.85% more than controls on average) was strikingly similar to the reduction in visits by the plucked males (9.87% less than controls on average) (Figure 2), moreover, the sum of the male and female visits did not significantly differ with treatment ($F_{1,51.02}$ = 0.001; p = .98). This suggests that females in the plucked group fully compensate for their male's reduced visitation. The number of visits were also significantly affected by brood size with more visits in nests with larger broods ($F_{1,36.09}$ = 9.99; p < .01; slope = 30.08 ± 9.45), hatching date with earlier nests having more visits ($F_{1,27.22}$ = 7.41; p = .02; slope = −4.33 ± 1.58), and chick age with a lower number of visits at chick day 11 than day 10 ($F_{1,101.91}$ = 12.78; p < .01; day 11 = 21.67 ± 6.06 fewer visits than day 10).

Finally, there was no significant effect of treatment on the proportion of caterpillars and spiders taken to the nest (caterpillars: PBtest = 0.52, p = .50; spiders: PBtest = 0.04, p = .86), neither a date nor sex effect (Table S1).

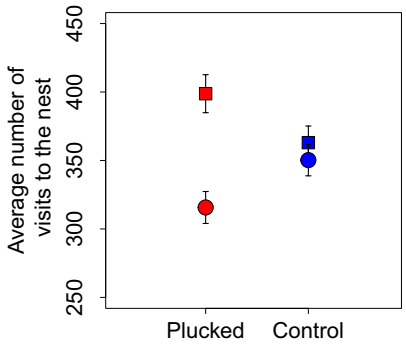


FIGURE 2 Average number of visits to the nest (with *SE*s) by males (circles) and females (squares) in plucked (red) or control (blue) treatments. The interaction between sex and treatment is significant

3.3 | Flight performance

We tested the effect of wing gaps on flight performance using 26 plucked and 29 control birds. In total, we recorded 303 flights in the vertical flight tunnel. We used 147 of the recorded manoeuvres for our analysis (Figure 1c), as these were scored as "straight" flights and they were from birds that were not excluded from the analysis as mentioned above. Every individual had at least a single straight flight sequence. Based on the analysis of these straight trajectories, we found that the control birds had an average flight speed of $U_{mean,control}$ = 2.52 ± 0.23 m/s ($M \pm SD$, n = 22), of which the vertical velocity component was $w_{mean,control}$ = 2.36 ± 0.24 m/s. At an average wingbeat frequency of $f_{mean,control}$ = 19.9 ± 1.41 Hz, this resulted in a displacement per wingbeat of $D_{mean,control}$ = 0.13 ± 0.01 m, and a vertical displacement per wingbeat of $D_{z,mean,control}$ = 0.12 ± 0.01 m. Flight performance analyses were also conducted correcting the components by the birds body mass and effects were very similar when correction was not made.

Males in the plucked group had a 5% smaller displacement per wingbeat ($D_{mean,plucked}$ = 0.12 ± 0.01 m) than the control group ($F_{1,37.26}$ = 5.31, p = .03, Figure 3a,b). Our results suggest that the plucked animals partially compensate for this reduction in distance travelled per wingbeat by increasing wingbeat frequency 2.5% compared to the control group ($f_{mean,plucked}$ = 20.4 ± 1.45 Hz, Figure 3a), and as a result plucked birds flew 2.0% slower than that of the control group ($U_{mean,plucked}$ = 2.47 ± 0.29 Hz, Figure 3c), although differences in frequency ($F_{1,38.15}$ = 1.61, p = .21, Figure 3c,d) and flight speed alone in simple regression ($F_{1,38.5}$ = 1.05, p = .31, Figure 3e) were not significant.

We did find a significant effect of the interaction between treatment and second moment of wing area on flight velocity ($F_{1,36.26}$ = 6.14, p = .02, Figure 3f). This shows that birds in the control group had the expected relation of a naturally smaller second moment of wing area leading to a lower upward flight velocity (slope = 0.17 ± 0.10). In contrast, for the males in the plucked group, these dynamics were reversed such that the flight speed increases with a decreasing second moment of area of the wing (slope = −0.21 ± 0.11). This suggests that treatment birds behaviourally overcompensate in response to a reduction in second moment of wing area caused by plucking the feathers. This compensation might be achieved by a concomitant increase in wingbeat frequency (Figure 3b), but a similar interaction between treatment and second moment of wing area was only borderline different ($F_{1,35.78}$ = 3.38, p = .07, Figure 3c, Table S3), thus other kinematics changes such as an increase in wingbeat amplitude or angle-of-attack might also be involved (Muijres, Bowlin, Johansson, & Hedenström, 2012).

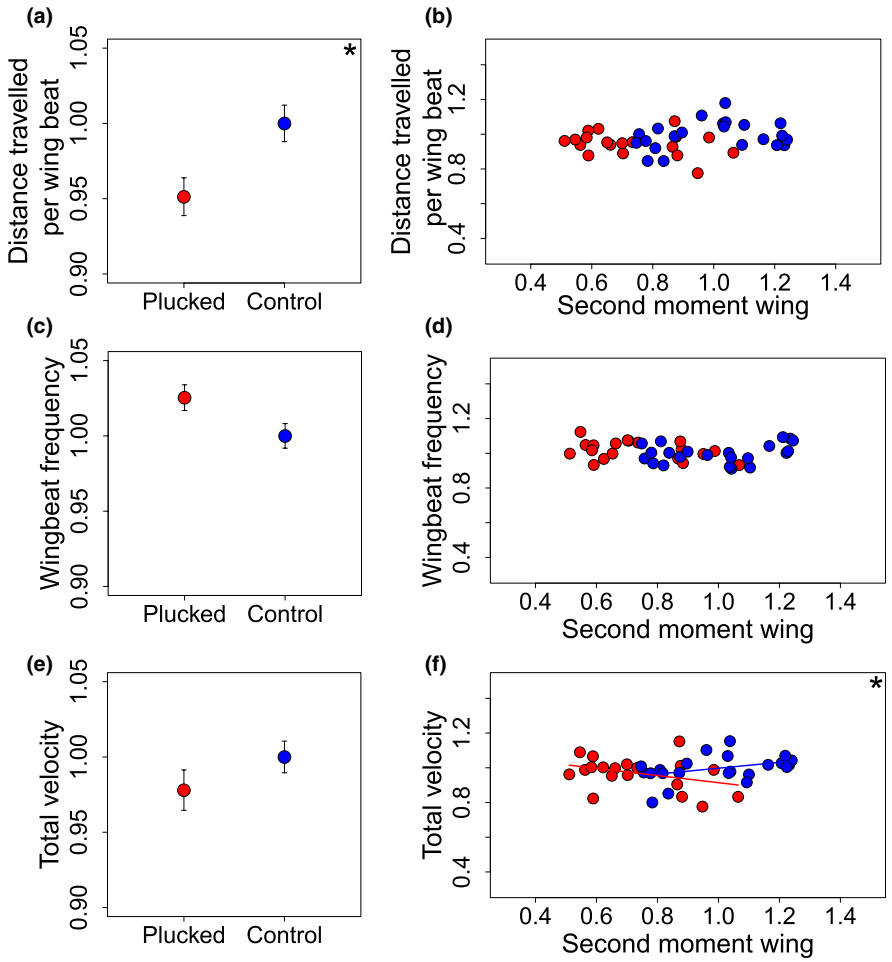


FIGURE 3 Flight performance metrics of males from plucked (red) or control (blue) treatments: (a, c, e) average of all individuals per treatment (with *SE*s) or (b, d, f) plotted in relation to the second moment of wing area with each dot representing one individual. (a, b) distance travelled per wing beat, with a significant treatment effect; (c, d) mean wingbeat frequency; (e, f) total velocity, with a significant interaction between treatment and second moment of wing. To simplify the comparison, all variables were non-dimensionalised by dividing each value by the average value for the control group

3.4 | Oxidative stress

There was no effect of treatment on ROMs or OXY (Table S5). Variation in these oxidative damage measurements was also not related to second moment of wing area or sampling date (Table S5).

3.5 | Carry-over effects

There was no effect of treatment on the probability of either male or female local survival and also there was no effect on the female laying date or clutch size in the year after the experiment (Table S6). Effects of previous year brood size and hatching date of the chicks were also not significant. Geolocator deployment also did not affect the probability of male local survival (Table S6).

In the case of female local survival probability, although not significant, the proportion of surviving females from the control group was larger than plucked, thus, in the expected direction (control = 11 out of 28, 39%; plucked = 6 out of 25, 24%). A significant difference with this sample size would only be apparent if we had a much more extreme difference in return rates (i.e. only 2 out of 25 in plucked group vs. 11 out 28 in controls). In males, this difference in return rates was also not significant, but was much smaller than the one observed for females (control = 7 out of 28, 25%; plucked = 7 out of 25, 28%), even if we restricted our sample to birds that did not carry geolocators (control = 1 out of 4, 25%; plucked = 6 out of 20, 30%).

4 | DISCUSSION

Our results suggest that male pied flycatchers with experimentally induced wing gaps suffer from a lower flight performance as the removal of the second and third primary flight feather led to a 5% reduction in travel distance per wingbeat in upward directed flight. This reduction in flight performance might result in increased energetic costs of foraging and commuting flight, prey capture success or predator avoidance and would be expected to be detrimental if occurring during chick provisioning. However, these same birds with this simulated moult did not have a decline in their condition in terms of oxidative damage, moult onset or body mass while rearing their chicks. This is consistent when these plucked males prioritise their future reproduction by reducing parental care, as they did: they reduced the number of visits to the nest. Interestingly, this reduction in male parental care did not affect their current breeding success, as their chicks did not differ in their fledging mass or fledgling success. This could be explained by the compensatory increase in parental care of the females. Finally, while local survival was almost the same for males from both groups, a lower proportion of females paired with a plucked male returned to breed in the following spring in relation to controls (although not significant). Therefore, although males had a reduced flight performance, it seems that the females paid the costs of the males` moult-breeding overlap.

As mentioned before, although many studies explored moult-breeding trade-off, just a few of them tested the direct effects of moult-breeding overlap (Table 1). Thus, a more direct comparison with our results is only possible with the study of Hemborg and Lundberg (1998) who reported a decrease in body condition of males, females and chicks, which we did not find in the present study. Our results for female return rates are in the same direction as Hemborg and Lundberg (1998) found for males, but are not significant (24% of females in the plucked group returned to our study site as opposed to 39% in the control group). This may be due to the fact that in their study, both males and females were handicapped, increasing the effect, while we only handicapped males. Females do not moult while breeding as often as males and costs of overlapping these two stages may be much higher in females (Jenni & Winkler, 1994). Thus, it is likely that males in our study were only able to maintain their condition and still be successful in fledging their broods because of the compensatory capacity of the females. Moreover, it is important to remember that the moult involves other aspects than wing gaps, such as physiological changes (Jenni & Winkler, 1994); therefore, our simulation of a moult-breeding overlap may be milder than the real moult.

Here, we decided to use the components of oxidative stress as an indirect measure of increased activity and effort of the animals due to an inefficient flight during the energetically demanding chick feeding stage. There is some debate on how oxidative stress components and metabolic rates would be related (Salin et al., 2015), however, we expected that if the males from the plucked group were working harder, this would reflect in higher levels of ROS (Monaghan et al., 2009). But, in accordance to the nest visitation results, there were no differences in ROS between the two groups. This suggests the two groups of males did not differ in their effort. However, since the oxidative stress status was measured 1 day after the flight trial, birds could have recovered from the flight effort, thus this result is not conclusive.

We did observe that females in the plucked group visited their nests 10% more than females in the control group, suggesting they allocated more effort in parental care. Curiously, females still did not significantly differ in their return rates from their wintering grounds. It is possible that this was a limitation of our small sample size, but other studies also showed lower return rates for males but not females (Hemborg & Lundberg, 1998). Furthermore, it is possible that females raising their broods post-fledging alone (males were taken to the laboratory) were more prone to shift their efforts from current reproduction to future survival after fledging; thus redirecting the costs to the chicks. Since in our population it is common that half of the chicks only recruit 2 years after fledging (Visser et al., 2015), we did not have enough data to compare return rates of chicks. Thus, there could still be costs in terms of chick recruitment that our analysis did not detect.

The escape manoeuvres that we measured in the plucked birds (Figure 1c) were the result of a complex interaction between physical effects of wing gaps on flight performance in the form of a reduction in the distance travelled per wingbeat (Figure 3a), and a behavioural response in which the plucked birds overcompensate for the detrimental effects of moult on wing morphology (Figure 3f). The reduction in second moment of wing area as a result of our treatment, negatively affects the ability to produce aerodynamic forces (Muijres et al., 2016;

Weis-Fogh, 1973), and the plucked birds with lowest S_2 respond to this by flying at the highest escape speed (Figure 3f). Because of this overcompensation to a reduction in S_2, flight speed is not significantly different between the plucked and control group (Figure 3e). This behaviour might, therefore, lead to the fact that the reduction in flight performance in the plucked birds, and particularly that of the plucked birds with the largest reduction in S_2, is masked to the outside observer such as the experimenter or more importantly a predator.

An additional curious result of our experiment is the fact that variation in flight performance (assumed as distance gained per wing beat) also relates to the variation in the number of visits, but, again, the direction of the relation is the opposite between plucked and controls, hinting at a different behaviour of the plucked birds (see Appendix S1, Table S7, for more information).

The flight trials had to be performed in captivity and thus should be extrapolated with care to the field situation (Lind, Gustin, & Sorace, 2004). However, they are reliable to interpret the effects of wing gaps between the groups since all individuals were treated in the same way, from capture to housing, with the same ad lib food and time spent in captivity prior to the trials, therefore differences between plucked and control individuals should be attributed to the experimental effects alone.

Overall, our results suggest that, when faced with the trade-off between current and future reproduction, male European pied flycatchers will prioritise their future reproduction. Although they do suffer flight costs due to moult, when they overlap moult and breeding, they do not suffer high fitness costs, since they are still able to maintain their body condition and successfully raise their chicks. Females, in contrast, seem to pay this cost by prioritising their current reproduction, trying to compensate for their males' reduced effort and suffer from survival costs.

The recent increase in temperature caused by climate change causes changes in the timing of annual cycle stages but not at the same rates (Both & Visser, 2001; Crozier et al., 2008; Ozgul et al., 2010; Moyes et al., 2011; Tomotani, van der Jeugd, et al. (Submitted)). This means that this already short interval between breeding and moult may be either further reduced depending on how fast breeding and moult shift Tomotani, van der Jeugd, et al. (Submitted). Earlier breeding, on the other hand may also allow a longer duration of moult if departure does not change. By overlapping moult and breeding male flycatchers may benefit from an earlier departure and/or from more time to grow a high-quality plumage, serving as a buffer against these time pressures (Helm & Gwinner, 2006), but at the expense of the females' survival. Moreover, further extending the moult into the breeding stage may be constrained by the increasing flight costs of the later moult stages (Hemborg et al., 2001). Therefore, unequal shifts in annual cycle stages caused by climate change may force males and females to face increasing costs of moult-breeding overlap depending on how fast different annual cycle stages shift in relation to one another.

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AUTHORS' CONTRIBUTIONS

B.M.T., F.T.M. and M.E.V. conceived the ideas and designed methodology; B.M.T. conducted the experiment; B.M.T., F.T.M. and J.K. processed the data; S.C. ran the laboratory assays; B.M.T., F.T.M. and M.E.V. analysed the data; B.M.T. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data supporting this manuscript are available at the Dryad Digital Repository https://doi.org/10.5061/dryad.d3b3t (Tomotani, Muijres, Koelman, Casagrande, & Visser, 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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